NTP
National Toxicology Program

Toxicology and Carcinogenesis Studies of α-Methylstyrene in F344/N Rats and B6C3F$_1$ Mice

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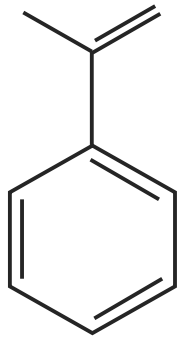
Background and Nomination

- Intermediate chemical used in production of acrylonitrile-butadiene-styrene resins and copolymers, modified polyester and alkyl resins, solvent for plastic industry
- Products - floor tiles, adhesives, floor polishers, plastics, water heaters, household machinery, waxes, inks, and other products
- Nominated by the EPA for toxicological evaluation and genotoxicity studies
  - High production volume
  - Limited toxicology information
- Structurally similar to styrene



- Studies performed: 3-month, 2-year, genotoxicity studies

Morgan *et al*. (1999) Toxicol Sci **47**, 187-194

α-Methylstyrene 2-Week Studies

- Whole body inhalation exposure for approximately 2 weeks
- Male and female F344 rats (up to 1000 ppm)
  - Increased liver weights at highest doses without corresponding histopathology
  - Hyaline droplet accumulation in male kidney at ≥250 ppm
- Male and female B6C3F$_1$ mice (up to 1000 ppm)
  - No adverse effects up to 500 ppm
  - Sedation and several deaths occurred in all groups of females
  - Increased liver and decreased spleen weights, no observed histopathology

3-Month Studies

- Whole body inhalation exposure
- Male and female F344 rats and B6C3F$_1$ mice (n=10)
- Exposure concentrations of 0, 75, 150, 300, 600, 1000 ppm
- 6 hours a day, 5 days a week, 14 weeks

3-Month Study Results - Rats

Males

	Control	75 ppm	150 ppm	300 ppm	600 ppm	1000 ppm
Hyaline droplet accumulation	9 (1.1)	10 (1.2)	10 (1.3)	10 (1.1)	10 (1.8)	10 (1.7)
α2u-Globulin (nmol/g tissue)	195	349*	497**	689**	724**	749**
α2u-Globulin (ng/µg sol prot)	81	115	119*	161**	176**	167**
Labeling index (%)	2.34	3.03	3.08**	3.35**	3.05**	3.94**

n = 10; * $p < 0.05$, ** $p < 0.01$

- Increased kidney weights in males (1000 ppm) and females (≥600 ppm)
- Increased liver weights in males (≥150 ppm) and females (≥600 ppm)
  - No corresponding histopathological changes

3-Month Study Results - Mice

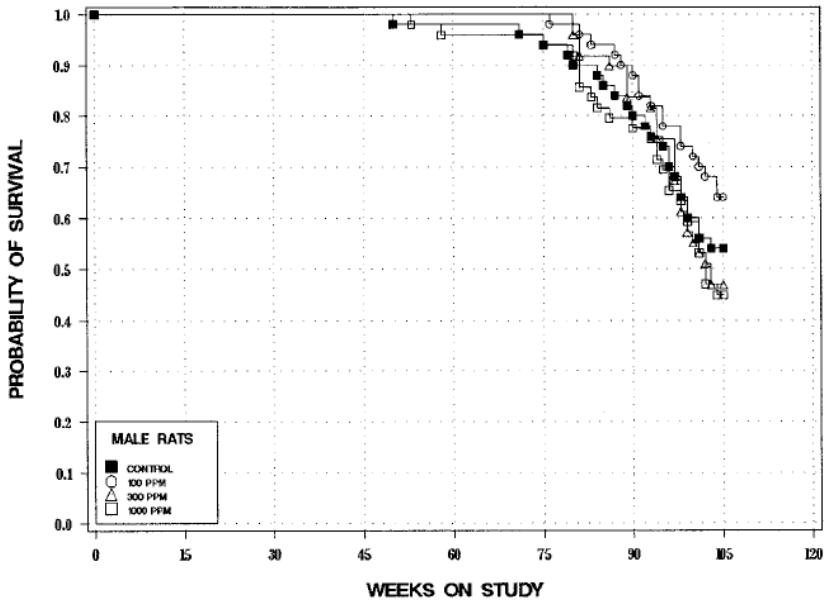
- Two early deaths in the 1000 ppm female group
- Ataxia in both sexes and moderate to severe sedation in males at 1000 ppm
- Terminal BW decreased in males (≥600 ppm) and females (75, 300, and 1000 ppm)
- Overall BW gain decreased in both sexes (≥300 ppm)
- Exposure concentration-dependent increased liver weights in both sexes
- Minimal to mild centrilobular hypertrophy in both sexes (≥600 ppm)
- Increased estrous cycle length in ≥600 ppm females

Chronic Study Design

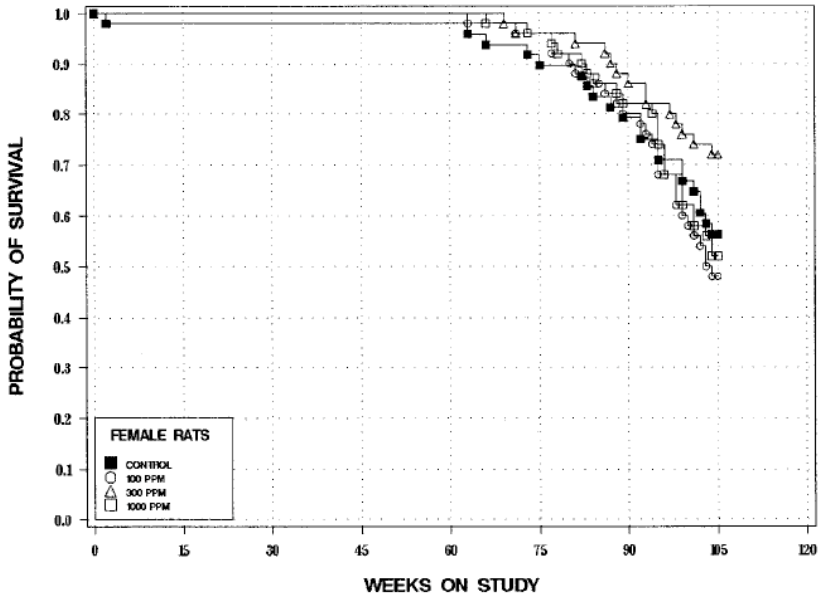
- Whole body inhalation exposure
- Male and female F344 rats
  - Exposure concentrations of 0, 100, 300, and 1000 ppm
  - 6 hours a day, 5 days a week, 105 weeks
- Male and female B6C3F$_1$ mice
  - Exposure concentrations of 0, 100, 300, and 600 ppm
  - 6 hours a day, 5 days a week, 105 weeks

No Exposure-Related Effects on Survival in Rats

Males

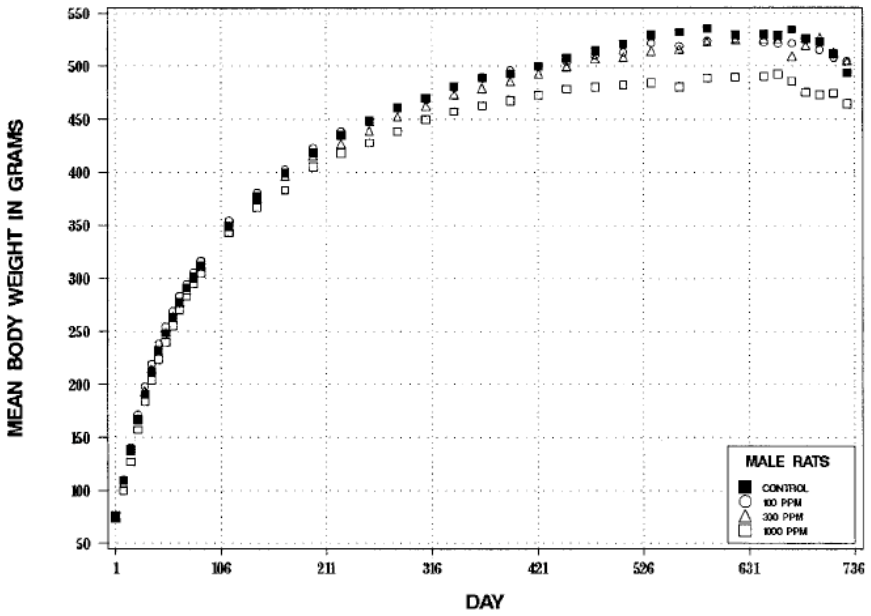


Females

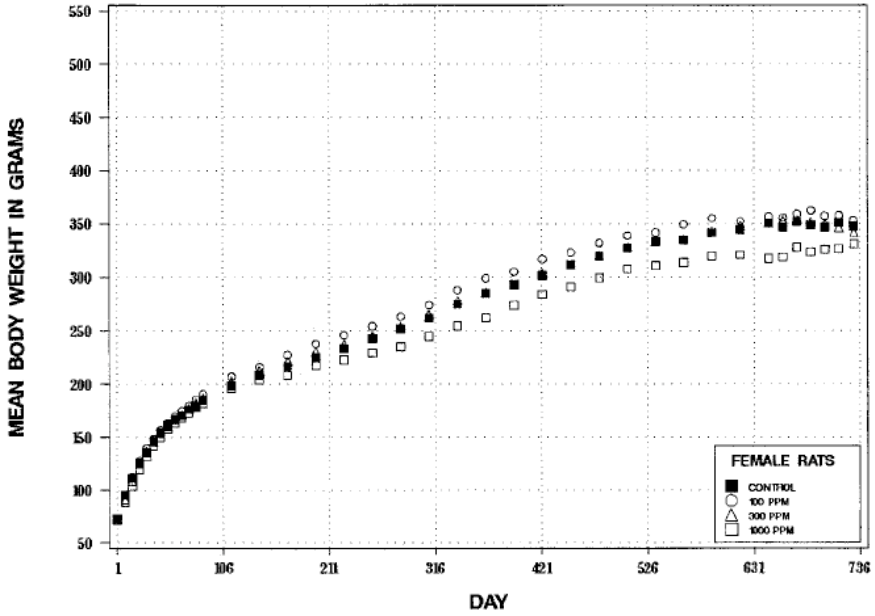


Decreased body weights in 1000 ppm male and female rats

Males



Females



Incidence and Severity of Kidney Lesions in Rats

Males

Single Sections				
	Control	**100 ppm**	**300 ppm**	**1000 ppm**
Tubule hyperplasia Papilla mineralization Nephropathy	0 12 (1.1) 41 (2.2)	0 16 (1.0) 46 (2.3)	0 10 (1.0) 46 (2.4)	0 33** (1.4) 45 (2.4)
Renal tubule adenoma Adenoma or carcinoma	0 0	0 0	1 2	0 2
Single and Step Sections				
	Control	**100 ppm**	**300 ppm**	**1000 ppm**
Tubule hyperplasia	1 (1.0)	0	1 (1.0)	4 (2.3)
Renal tubule adenoma Adenoma or carcinoma	1 1**	2 2	2 3	5 7*

Females

	Control	**100 ppm**	**300 ppm**	**1000 ppm**
Papilla mineralization	1 (1.0)	6 (1.0)	8* (1.0)	7* (1.0)

n=49-50, * $p < 0.05$, ** $p < 0.01$

Additional Findings in Rats

Males

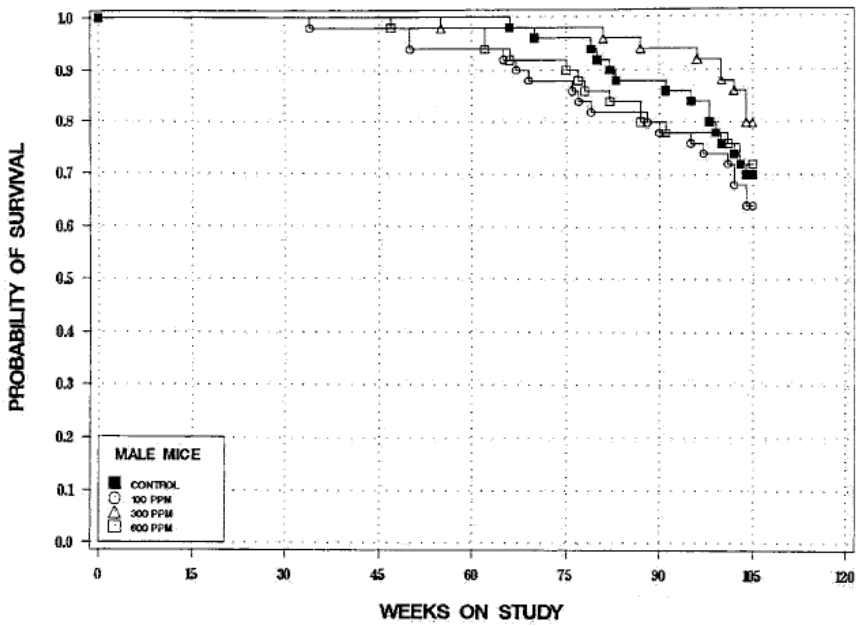
	Control	100 ppm	300 ppm	1000 ppm
Mononuclear Cell Leukemia	26* (52%)	32 (64%)	29 (58%)	38* (76%)

n=50, * p < 0.05; Historical control incidence:188/399 (47%); Historical control range: 32-66%

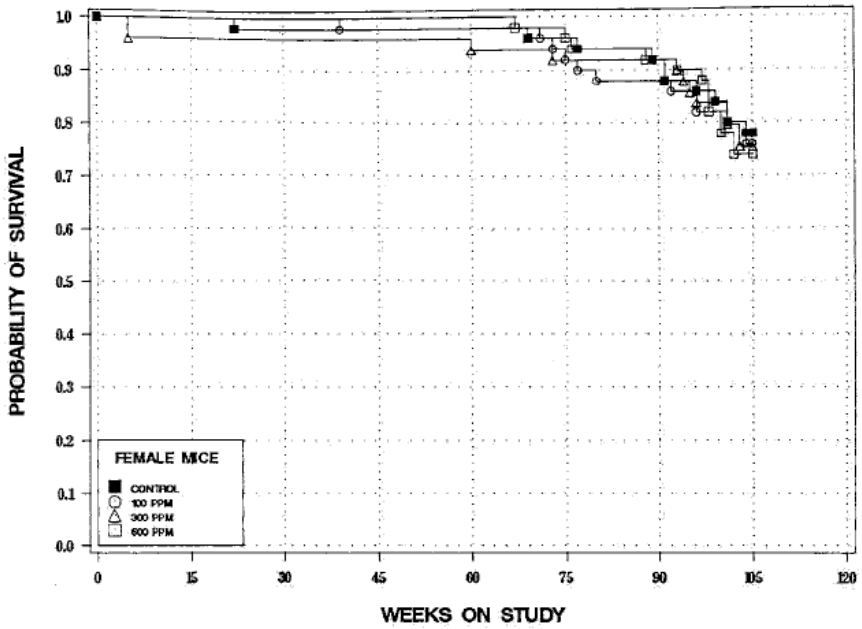
- Exposure concentration-related increased incidences of degeneration and basal cell hyperplasia in the olfactory epithelium of the nose

No Exposure-Related Effects on Survival in Mice

Males

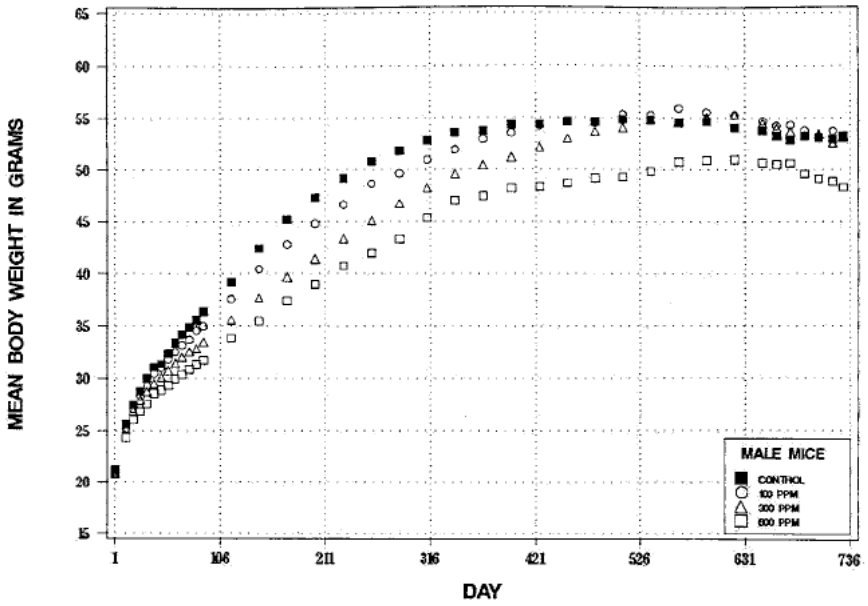


Females

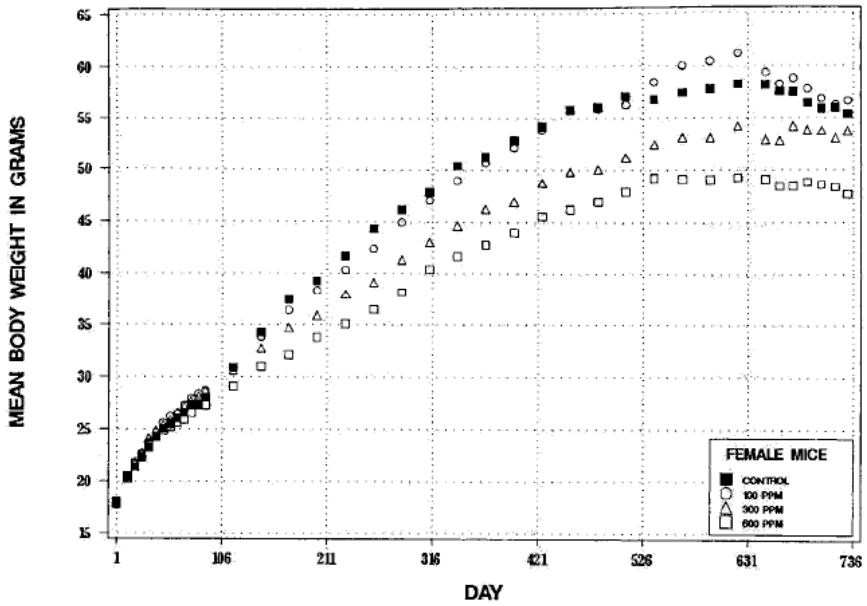


Decreased body weights in 600 ppm male and female mice

Males



Females



Incidence of α-Methylstyrene-induced Liver Neoplasms in Mice

Males

	Control	100 ppm	300 ppm	600 ppm
Hepatocellular adenoma	24	27	27	25
Hepatocellular carcinoma	10	12	11	17
Combined	28	36*	33	37*

Females

	Control	100 ppm	300 ppm	600 ppm
Eosinophilic foci	2	5	7	12**
Hepatocellular adenoma	10*	20*	21**	23**
Hepatocellular carcinoma	3**	9	6	18**
Combined	13**	26**	24*	33**

n=50, * $p < 0.05$, ** $p < 0.01$

Incidence and Severity of Nasal Lesions in Mice

Males

	Control	100 ppm	300 ppm	600 ppm
Metaplasia, olfactory epithelium glands	6 (1.2)	47** (2.7)	49** (3.0)	49** (3.0)
Hyperplasia, olfactory epithelium	4 (1.0)	50** (2.8)	50** (3.0)	50** (3.1)
Atrophy, olfactory epithelium	0	2 (2.5)	8** (1.8)	12** (1.7)

Females

	Control	100 ppm	300 ppm	600 ppm
Metaplasia, olfactory epithelium glands	2 (1.0)	49** (2.7)	47** (3.0)	50** (3.0)
Hyperplasia, olfactory epithelium	3 (1.0)	49** (2.9)	50** (2.9)	50** (3.0)

n=50, * $p < 0.05$, ** $p < 0.01$

Additional Findings in Mice

- Increased incidence and severity of nephropathy in the kidney of the 600 ppm females
- Increased incidence of hyperplasia of the forestomach epithelium in the 300 and 600 ppm males

Genetic Toxicology

- No induction of gene mutations in *Salmonella typhimurium* strains TA97, TA98, TA100, or TA1535 in either the presence or absence of S9 liver fraction
- Significant increase in sister chromatid exchanges in cultured Chinese hamster ovary cells in the presence of S9 only
- No induction of chromosomal aberrations in cultured Chinese hamster ovary cells in either the presence or absence of S9
- No increase in the frequency of micronucleated erythrocytes in peripheral blood of male mice exposed by inhalation for 3 months
- Significant increase in micronucleated erythrocytes in peripheral blood of female mice exposed by inhalation for 3 months

Evidence for Carcinogenic Activity

- *Some evidence* in male rats based on increased incidences of renal tubule adenomas and carcinomas (combined)
  - Increased incidence of mononuclear cell leukemia in the 1000 ppm males may have been related to *α*-methylstyrene exposure
- *No evidence* in female rats
- *Equivocal evidence* in male mice based on marginally increased incidences of hepatocellular adenoma and carcinoma (combined)
- *Clear evidence* in female mice based on increased incidences of hepatocellular adenomas and carcinomas

PBPK Model

- Derived from published models developed for styrene, structurally similar to α-methylstyrene
- Model simulations for urinary excretion and tissue concentrations provide good fit for experimental data
- Although biological effects differ between styrene and α-methylstyrene, model suggests ADME is similar